The Dangers of Mitochondrial DNA Heteroplasmy in Stem Cells Created by Therapeutic Cloning

Grant Award Details

The Dangers of Mitochondrial DNA Heteroplasmy in Stem Cells Created by Therapeutic Cloning

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Investigator:

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Institution:	University of California, Irvine
Type:	PI

Human Stem Cell Use: Embryonic Stem Cell, Other

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Grant Application Details

Application Title: The Dangers of Mitochondrial DNA Heteroplasmy in Stem Cells Created by Therapeutic Cloning

Public Abstract: n therapeutic cloning, a patient's cell is combined (fused) to an enucleated donated egg (oocyte) from an unrelated woman or from another animal. It is hoped that cellular factors in the egg cytoplasm will reprogram the patient's cell nucleus making it capable of generating replacement cells for the patient's body. Thus, if a patient is suffering from Parkinson Disease due to loss of brain cells, these cells could be replaced with differentiated, individualized, nuclear-transplantation, embryonic stem (ntES) cells. While this strategy should generate ntES cells with the patient's nuclear DNA (nDNA), it overlooks the fact that another part of the cell, the mitochondrion, also has DNA, the mitochondrial DNA (mtDNA). While the nDNA contains the blueprints for assembling the structure of the cell and body, analogous to carpenter's plans for a house; the mtDNA contains the blueprints for the cellular electrical system, the wiring diagram of the house. It is universally agreed that mixing the nDNAs from two different cells would be destructive, yet the potentially disastrous effects of mixing different mtDNAs has been overlooked. In electricity, randomly mixing the components of two different integrated electrical circuits will result in short circuits. The same appears to be true for the cell. In mice in which we artificially mixed two mtDNAs, the resulting mice aged and died prematurely, had a striking increased frequency of cancer, and an increased mtDNA mutation rate. Moreover, in human studies, the accumulation of mtDNA mutations has been associated with aging and the development of cancer. Therefore, to document what happens to the mtDNAs during the creation and growth of htES and hES cells, we propose to create ntES cells by fusion of human cells to enucleated rabbit eggs and then to monitor the fate of the human and rabbit mtDNAs. We will also determine if the mitochondria and mtDNAs of hES cells can influence the differentiated state of tissue cells, investigate the nature and extent of mtDNA mutations that accumulate in ES cells, and determine if mixing different mtDNAs in cells is deleterious. Then we will determine the effects of these various mtDNA genetic factors on the power output of the mitochondria and its effects on the ES cell's capacity to differentiate and potentially to form tumors. Finally, we propose to develop a series of procedures to control the origin and nature of the mtDNAs in ntES and hES cells and to use changes in mitochondrial function to regulate ntES and hES cell growth and regulation. It is our hope that by utilizing mitochondrial biology and genetics it may be possible to develop strategies for creating individualized stem cells without using donated oocytes.

Statement of Benefit to California:	Prolonged cell culture of human Embryonic Stem Cells (hESCs) frequently results in the loss of the cell's capacity to differentiate on command into well differentiated cells. This eliminates their utility for generating replacement cells for use in cell replacement therapy to repair damaged tissues and organs within the body. The reason for this loss of developmental capacity by the hESCs is currently unclear, but we believe that a major factor contributing to the decline in the therapeutic value of hESCs is the accumulation of deleterious mutations in the mitochondrial DNA (mtDNA) of the cultured hESCs. The mtDNAs are located in the mitochondria which are organelles in the cytoplasm of the human cell. The mitochondria are responsible for generating most of the energy used by the cell and as a toxic by-product, the mitochondrial generate most of the endogenous reactive oxygen species (ROS). The mtDNA encodes key elements of the mitochondrial energy generating apparatus, and since ROS is a mutagen, the mtDNA is highly prone to acquiring mutations in these energy genes. These mutations then inhibit mitochondrial energy production which also results in increased ROS production. Increased mitochondrial ROS production stimulates the cell growth, so the cells with the mutant mtDNAs out grow the normal cells. However, the more rapidly growing cells with the mutant mtDNAs also have reduced mitochondrial energy production, which together with the increased ROS production, inhibits the developmental capacity of the hESC. In this research, we propose to establish that deleterious mtDNA mutations do in fact accumulate in hESCs over time and that they play an important role in the loss of the developmental potential of hESC cells. If we can confirm that this is a fact, then we should be able to greatly increase the therapeutic potential of hESCs by developing procedures for protecting the mtDNA of the hESCs from oxidative damage. This might be accomplished by growth of hESCs in the presence of mitochondrially target antioxidants. Furthermore, cells that had lost their developmental potential might be revitalized by simply replacing the damaged mtDNAs with good mtDNAs using our trans-mitochondrial cybrid technique. Thus, the proposed research has the potential of greatly increasing the therapeutic potential of all hESCs that will be developed in the State of California.